

Effects of insulin on long-term potentiation in hippocampal slices from diabetic rats

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Abstract

Aims/Hypothesis. Cognitive deficits occur commonly in diabetic patients. It is unclear whether these impairments result from hypoglycaemia during intensive insulin therapy, or from the diabetes itself. The aim of this study was to examine if impaired energy utilization resulting from insulin deficiency contributes to impaired long-term potentiation (reflecting impaired synaptic plasticity). As long-term potentiation is considered a candidate cellular mechanism underlying learning and memory, understanding how diabetes alters long-term potentiation may provide insight into mechanisms producing cognitive deficits in diabetes.

Methods. Electrophysiologic recordings were used to study long-term potentiation in the CA1 region of hippocampal slices from healthy rats and rats with streptozotocin-induced diabetes.

Results. Long-term potentiation was difficult to induce in slices from diabetic rats in standard recording buffer (contains 10 mmol/l glucose). In slices from diabetic rats, increasing extracellular glucose failed

to recover long-term potentiation induction, but 10 mmol/l pyruvate added to standard buffer enabled long-term potentiation induction. Moreover, incubation of slices from diabetic rats with insulin enabled long-term potentiation induction in standard buffer. Acute administration of streptozotocin alone did not impair long-term potentiation in slices from healthy animals, and changing extracellular glucose concentrations over the range of 5 mmol/l to 30 mmol/l did not alter long-term potentiation in slices from control rats.

Conclusions/interpretation. These observations suggest that impaired energy utilization from insulin deficiency, rather than the accompanying hyperglycaemia, impair long-term potentiation in diabetes. Impaired hippocampal synaptic plasticity could contribute to learning and cognitive impairment in diabetic patients. [Diabetologia (2003) 46:1007–1012]

Keywords Diabetes, streptozotocin, hyperglycaemia, hippocampus, synaptic plasticity, pyruvate, glucose utilization.

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Abbreviations: LTP, long-term potentiation; STZ, streptozotocin; AD, Alzheimer's type dementia; CNS, central nervous system; ACSF, artificial cerebrospinal fluid; PND, postnatal date; PS, population spike; CSF, cerebrospinal fluid; LTD, long-term synaptic depression.

Poor academic performance in diabetic children and memory impairment in adults with diabetes are viewed as increasing public health concerns. These problems are typically thought to result from hypoglycaemic attacks [1, 2], which occur more frequently during intensive insulin therapy [3]. Although insulin-induced hypoglycaemia could contribute to learning problems [4], one recent prospective study did not find a relationship between severe hypoglycaemic events and cognitive impairment [5], raising the possibility that the diabetic condition alone might adversely affect learning and memory. Experimental evidence supporting an independent effect of diabetes upon

brain function comes from studies showing that diabetic rats show memory impairment in the absence of insulin treatment or hypoglycaemia [6].

Experimental diabetes can be induced in rodents by treatment with streptozotocin (STZ), an agent that selectively destroys insulin-producing pancreatic beta cells. Rats treated with STZ show impairments in long-term potentiation (LTP) of hippocampal synaptic transmission, a form of synaptic plasticity thought to be involved in certain forms of memory processing. STZ-treated rats also show impaired performance in the Morris water-maze, a behavioural task that tests hippocampal-dependent spatial learning [6]. Previous studies suggest that hyperglycaemia is a principal cause of LTP impairment in STZ-treated rats because insulin therapy started immediately after STZ injection prevents the problem [6]. However, another study [7] found that hyperglycaemia induced by STZ paradoxically facilitates LTP induction in aged rats. As LTP induction is sensitive to changes in energy utilization [8, 9], it is possible that aging-induced changes in glucose use affect LTP induction. It has been suggested that Alzheimer's type dementia (AD) might represent a diabetes-like condition in the central nervous system (CNS) in which neuronal glucose utilization is impaired [10]. Consistent with this, poor glucose utilization is observed in the brains of AD patients [11, 12]. Furthermore, the beta amyloid protein that is involved in the pathophysiology of AD has been found to inhibit neuronal glucose utilization [13]. These observations raise the possibility that the LTP inhibition and memory impairment observed in rodents treated with STZ might not simply reflect the effects of hyperglycaemia or related complications. It is possible that poor energy utilization with an overabundance of glucose in the CNS results in cognitive dysfunction. Using hippocampal slices prepared from STZ-treated and healthy control rats, we examined possible mechanisms underlying changes in synaptic plasticity in diabetes.

Materials and methods

Animals. A single intraperitoneal (ip) injection of 60 mg/kg STZ (Sigma Chemical, St. Louis, Mo., USA), dissolved immediately before administration in 50 mmol/l citrate buffer, was given to male rats at postnatal day (PND) 30. All animals were allowed free access to water and laboratory food. Urine glucose was checked by Chemstrip uGK (Boehringer Mannheim) every 3 days until the time of hippocampal slice preparation. In some rats, blood and cerebrospinal fluid (CSF) were sampled prior to decapitation and a glucose oxidase method was utilized for assay of glucose. In control rats, the CSF/blood glucose ratio was greater than 0.6 [15].

Hippocampal slice preparation. Transverse slices were prepared from the septal half of the hippocampus using standard techniques [14]. Albino rats (44±2 days old) were anaesthetized with halothane and decapitated. The hippocampi were

rapidly dissected at 4 to 6°C and cut into 500 µm slices using a Campden vibrotome (Campden Instruments, Sileby, Loughborough, U.K.). Slices were then kept in artificial cerebrospinal fluid (ACSF) containing (in millimolar): 124 NaCl, 5 KCl, 2 MgSO₄, 2 CaCl₂, 1.25 NaH₂PO₄, 22 NaHCO₃, 10 glucose, bubbled with 95% O₂-5% CO₂ in an incubation chamber for at least 60 min at 30°C.

Electrophysiology. For synaptic experiments, slices were transferred to a submersion recording chamber where they were continuously perfused with ACSF (2 ml/min) at 30°C. Extracellular recordings were obtained from the pyramidal cell layer and dendritic region of CA1 using 5 to 10 MΩ electrodes filled with 2 M NaCl. During an experiment, the Schaffer collateral-commissural fibres were stimulated in stratum radiatum with bipolar electrodes and 0.1 to 0.2 ms constant current pulses at an intensity sufficient to evoke a 50% maximal population spike (PS) based on a baseline stimulus-response curve. Half-maximal stimulus intensities allow easy and reliable detection of changes in synaptic transmission. The initial slope of the EPSP was used for data analysis. The average slope of the EPSPs during a 20 min baseline recording period was used to normalize the EPSP slopes for comparison among slices. The EPSP slopes are shown as a percentage of the average baseline EPSP slope. LTP was induced using a single 1 sec tetanus at 100 Hz. Potentiation of the EPSP slope by more than 20% 60 minutes after tetanic stimulation was considered to represent successful induction and maintenance of LTP. Only a single slice from each hippocampus was used for each group of experiments. Values in the text represent means ± SE, unless otherwise noted. Chemicals were obtained from Sigma-Aldrich.

Statistical analysis. Data in the text and figures are expressed as means ± SEM. The Mann-Whitney U test and Student's *t* test were used for comparisons between groups. A *p* value of less than 0.05 was considered statistically significant.

Results

In nine of eleven rats treated with STZ, a diabetic state was established within 2 weeks as characterized by glycosuria and hyperglycaemia. Final blood glucose concentrations obtained at the time of preparation of hippocampal slices were 401±170 mg/dl (mean ± S.D., *n*=11). We excluded two rats from the study because they had normal blood glucose concentrations (<200 g/dl) at the time of dissection. Final blood glucose concentrations in the nine diabetic rats were 456±130 g/dl. Body weights were 120±63 g for diabetic rats and 163±26 g for control rats.

As reported by other investigators, LTP was difficult to induce in hippocampal slices from STZ-treated rats with 10 mmol/l glucose using a stimulation protocol that reliably generates LTP in slices from control rats (+5.1±2.6% change in EPSP slopes in diabetic rats, *n*=9 vs +41.3±6.3% increase in EPSPs in matched controls, *n*=5, *p*=0.001) [6]. This failure of LTP induction does not result from direct effects of STZ because acute administration of STZ (60 mg/l) for 30 min did not alter either basal synaptic transmission or induction of LTP (Fig. 1).

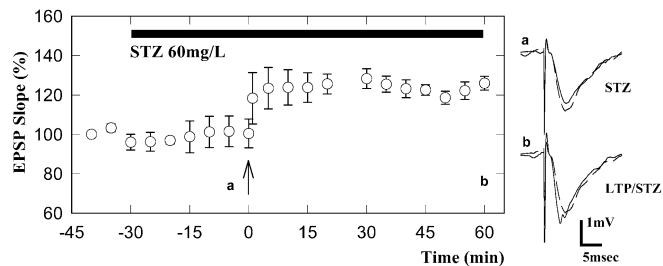


Fig. 1A, B. STZ does not acutely inhibit LTP induction in hippocampal slices from control rats. The graph shows the time course of change in field EPSP slope normalized with respect to initial baseline responses (100%). The traces to the right of the graph show representative EPSPs obtained 40 min before (*dashed traces*), just prior to tetanic stimulation (**A**) and 60 min after tetanic stimulation (**B**). The dashed trace is the baseline and the trace following treatment is shown as a continuous line. Basal synaptic transmission was not affected by 15 min perfusion of 60 mg/L STZ. Administration of STZ (filled bar) prior to tetanic stimulation (arrow) did not inhibit LTP induction

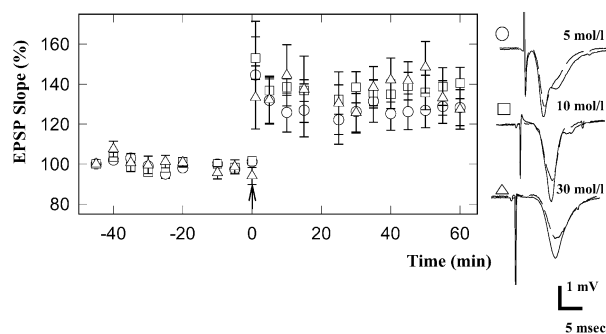


Fig. 2. High concentrations of glucose do not alter LTP induction in slices from healthy rats. LTP induction was not altered by raising extracellular glucose from 5 mmol/l (*open circles*) to 10 mmol/l (*open squares*) or 30 mmol/l (*open triangles*). The glucose concentration was changed from 10 mmol/l to 5 mmol/l or 30 mmol/l prior to tetanic stimulation. The traces to the right of the graph show representative EPSPs obtained 45 min before (*dashed lines*) and 60 min after tetanic stimulation (*solid lines*)

It is possible that the difficulty with LTP induction in diabetic rats results from excessive extracellular glucose. However, in hippocampal slices from healthy rats, changing the extracellular glucose concentration over the range of 5 to 30 mmol/l did not alter LTP induction (Fig. 2). As LTP is typically studied with 10 mmol/l glucose and diabetic rats have blood glucose concentrations that exceed this concentration, it is possible that 10 mmol/l glucose represents a relatively low glucose concentration in slices from diabetic rats. To examine this, the extracellular glucose concentration was increased to 30 mmol/l in slices from STZ-treated rats, a concentration comparable to blood glucose concentrations observed in these animals. Slices were exposed to 30 mmol/l glucose starting at the time of hippocampal slice preparation. De-

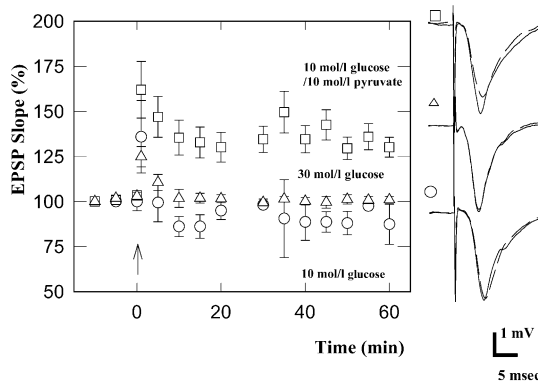


Fig. 3. Inhibition of LTP induction in slices from diabetic rats and the effects of pyruvate. In slices from diabetic rats, LTP was not induced by tetanic stimulation (arrow) with 10 mmol/l glucose (*open circles*) or 30 mmol/l glucose (*open triangles*). In the existence of 10 mmol/l pyruvate plus 10 mmol/l glucose, however, LTP was induced (*open squares*). The traces to the right of the graph show representative EPSPs obtained prior to (*dashed lines*) and 60 min after tetanic stimulation (*solid lines*)

spite high glucose concentrations in the ACSF, LTP still could not be induced in slices from STZ-treated rats (Fig. 3). These observations suggest that the failure of LTP induction in diabetic rats does not result from high glucose. In three of the nine diabetic rats studied, we sampled cerebrospinal fluid CSF before decapitation. CSF glucose concentrations were 151 ± 47 mg/dl, while blood glucose concentrations were 386 ± 54 mg/dl with a CSF/blood ratio of 0.39 ± 0.06 ($n=3$). This CSF/blood glucose ratio was lower than the ratio in control rats (0.6, $p=0.005$).

Although increases in extracellular glucose concentration did not permit LTP induction in slices from STZ-treated animals, it is possible that altered energy use is an important factor underlying the failure of LTP. As we previously observed that pyruvate can serve as an alternative energy substrate for LTP induction, we examined whether addition of pyruvate to the extracellular medium altered the generation of synaptic plasticity. Addition of 10 mmol/l pyruvate to 10 mmol/l glucose reliably allowed LTP in slices from STZ-treated rats ($+32.3 \pm 6.7\%$, $n=5$ By U test $p=0.005$ compared to glucose alone). However, LTP was not induced with 10 mmol/l or 20 mmol/l pyruvate alone (changes in EPSPs: $-1.8 \pm 6.2\%$, $n=3$; $+3.7 \pm 9.1\%$, $n=3$, respectively, data not shown). In control slices, 10 mmol/l pyruvate did not augment EPSPs with 10 mmol/l glucose, and LTP induced under these conditions was comparable to control LTP with 10 mmol/l glucose without pyruvate (changes in EPSPs: $+34.1 \pm 5.5\%$, $n=4$ pyruvate plus glucose; $36.5 \pm 4.1\%$, $n=5$ glucose without pyruvate, data not shown). These results indicate that the recovery of LTP in STZ-treated rats does not result from potentiation of synaptic responses or LTP by pyruvate, but rather that defective energy metabolism is likely to be involved in the failure to generate LTP. Although pyruvate can overcome

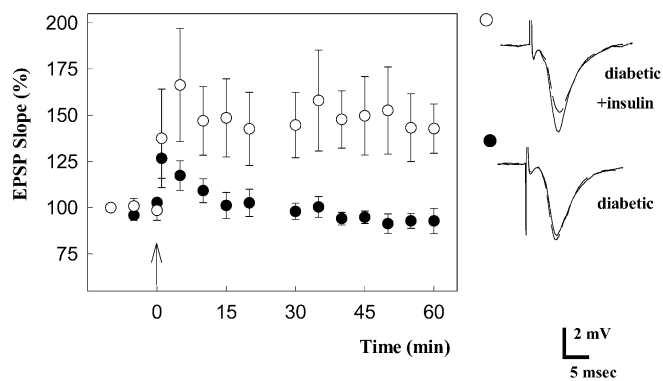


Fig. 4. Insulin overcomes the failure of LTP induction in slices from diabetic rats. In the existence of 10 mmol/l glucose, the failure of LTP induction in slices from diabetic rats (*filled circles*) was overcome by administration of 1 μ mol/l insulin (*open circles*). The traces to the right of the graph show representative EPSPs obtained prior to (*dashed lines*) and 60 min after tetanic stimulation (*solid lines*). Insulin was administered from the time of dissection and was continued throughout the experiments

this defect, recovery of LTP by pyruvate in STZ-treated rats remains dependent upon the presence of extracellular glucose.

Insulin deficiency or lack of insulin activity is likely to contribute to poor glucose utilization in STZ-treated rats. Increasing evidence suggests that insulin has direct actions in the CNS and modulates synaptic transmission. To examine the role of insulin deficiency in the failure of LTP induction, insulin was administered to slices starting at the time of dissection (Fig. 4). Slices were incubated with 1 μ mol/l insulin until the time of experiment (for ~2–3 h in total). We also continuously administered 1 μ mol/l insulin throughout these experiments. In the existence of insulin and 10 mmol/l glucose, LTP was consistently induced in slices from STZ-treated rats (changes in EPSPs: $+41.5 \pm 10.2\%$, $n=9$; $p=0.008$ by U test compared to no insulin). The degree of LTP was comparable to that in control slices incubated with 1 μ mol/l insulin (EPSP changes: $+44.6 \pm 8.3$, $n=5$, data not shown).

Discussion

Several studies have raised concerns about the effects of diabetes on memory and learning in children [2, 16, 17, 18]. Additionally, memory impairment has been observed in older adults with diabetes [19] and in diabetics with hypertension [20]. There is also evidence for an increased incidence of dementia in diabetic patients [21, 22, 23]. The mechanisms underlying these cognitive deficits remain uncertain with concerns being raised about the effects of insulin/hypoglycaemic therapies and the underlying illness itself [4, 24].

In efforts to develop an animal model for studying cognitive impairment in diabetic patients, it has been observed that rats treated with STZ develop a diabetes-like state and show altered synaptic plasticity and learning. LTP is widely considered to be a synaptic model for the information processing that underlies certain forms of memory and learning, and a correlation between changes in LTP and impaired performance in tests of spatial learning has been reported in a number of studies. Similarly, a correlation between diminished LTP induction or maintenance and poor performance in the spatial memory version of the Morris water-maze test has been observed in diabetic rats [25]. This suggests that understanding how defects in LTP occur could help to explain mechanisms involved in learning difficulties in diabetic patients.

In animals rendered diabetic by treatment with STZ, there are several potential mechanisms that could contribute to defects in LTP and learning. One possibility is that STZ directly impairs synaptic function. This seems unlikely because the failure of LTP induction has previously been correlated with the development of abnormal glucose concentrations rather than with STZ administration itself [6]. In our studies, acute administration of STZ to hippocampal slices did not impair either basal transmission or LTP induction in slices from healthy rats. Furthermore, intensive insulin treatment that normalizes blood glucose concentrations prevents the failure of LTP induction in STZ-rats, suggesting that it is the diabetic state itself that is responsible for the inhibition of LTP [6].

Although diabetes is primarily recognized on the basis of hyperglycaemia, STZ injections lead to insulin deficiency as well as poor glucose utilization. Thus, hyperglycaemia per se might not be the cause of LTP failure. Indeed, we observed that increasing glucose concentrations up to 30 mmol/l did not impair LTP in slices from healthy rats. Additionally, the standard glucose concentration (10 mmol/l) used for hippocampal slice recording is high relative to physiological levels in brain extracellular fluid and did not permit LTP induction in slices from STZ-treated animals. These observations suggest that the failure of LTP in slices from diabetic rats does not result from increased glucose concentrations. Interestingly, glucose concentrations in the CSF of diabetic rats were not as high as expected from the blood concentrations. Although the CSF/blood glucose ratio is normally about 0.67, the ratio is altered in diabetic patients and is often considerably less than 0.6 [26]. The relatively low CSF/blood glucose ratio in diabetic patients could result from down-regulation of blood-brain barrier glucose transporters [27, 28].

The role of insulin deficiency in the failure of synaptic plasticity is also important to consider. Although it has long been thought that the brain is an insulin insensitive organ [29], recent evidence suggests otherwise. Insulin can be transported from plasma into

brain through the blood brain barrier, achieving concentrations of about 3 pmol/l in the CNS [30, 31]. The existence of insulin receptors [32, 33] and GLUT-4, an insulin-sensitive glucose transporter [34, 35, 36], in the CNS supports the idea that insulin can exert direct actions in the CNS. Pre-incubation with insulin promotes survival of cultured neurons during glucose deprivation by facilitating glial glycogen synthesis [37]. In the hippocampus, insulin has several effects on cellular and synaptic function. In addition to effects on energy metabolism, insulin acutely inhibits spontaneous firing of pyramidal neurons [38], increases functional postsynaptic GABA receptors [39], and rapidly potentiates NMDA receptor-mediated EPSPs [40]. Furthermore, insulin-mediated tyrosine kinase activation phosphorylates NMDA receptors [41] and insulin can produce a form of long-term synaptic depression (LTD) in the CA1 region resulting from a decrease in membrane expression of AMPA-type glutamate receptors [42]. Although NMDA receptors are altered several months after STZ injection [43], this effect is unlikely to contribute to the failure of LTP induction in this study because we used rats 2 weeks after STZ injection. These observations make it reasonable to consider whether previously described effects of intensive insulin therapy on LTP induction result from direct effects of insulin in the CNS rather than correction of plasma glucose concentrations [25]. In our experiments, the ability to induce LTP in the presence of insulin in slices from STZ-treated animals suggests that insulin deficiency, rather than hyperglycaemia, contributes to the failure of LTP induction.

Although insulin has multiple actions in the CNS, our results suggest that insulin deficiency impairs energy metabolism and contributes to failure of LTP induction. We base this conclusion on the ability of pyruvate to promote recovery of LTP in slices from STZ-treated rats. Pyruvate, a monocarboxylate that can serve as an energy substrate in the CNS [44, 45], is transported into neurons through monocarboxylate transporters (MCTs) that are highly expressed in the hippocampus [46]. In slices from healthy rats, pyruvate alone supports LTP induction even without glucose [9]. However, pyruvate alone was not sufficient to support LTP induction in slices from STZ-treated rats, suggesting that its use as an energy substrate could also be impaired under diabetic conditions. Impaired glucose utilization in the brain [47] might be explained by depressed activity of glycolytic enzymes [48] or glucose transporters. In the hippocampus from diabetic rats, GLUT3, the neuron specific glucose transporter, shows increased protein conjugation by a lipid peroxidation product [49], possibly reducing neuronal glucose uptake.

A limitation of our study is that the duration of diabetes was only about 2 weeks. It will be important in future studies to address the question of whether insulin and pyruvate are effective in restoring LTP induc-

tion when the diabetic state is longer standing. In previous studies, systemic insulin failed to promote LTP induction when insulin administration was delayed following STZ injection [25].

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